15

Attorney Docket No. 10021301-1

METHODS AND APPARATUS FOR ANALYZING ARRAYS

20   ## BACKGROUND OF THE INVENTION

The present invention relates to apparatus and methods for conducting chemical and biological analyses using microarrays. More particularly, the invention relates to apparatus and methods for conducting and measuring the results of hybridization reactions involving linear microarrays of biopolymers wherein molecular probes on a

25   surface of a solid substrate selectively bind target molecules provided in a solution. The invention has utility in fields relating to biology, chemistry and biochemistry. The invention has particular application to the area of analyzing the results of hybridization reactions involving nucleic acids and proteins in small chambers such as capillary chambers.

30   Determining the nucleotide sequences and expression levels of nucleic acids (DNA and RNA) is critical to understanding the function and control of genes and their relationship, for example, to disease discovery and disease management. Analysis of genetic information plays a crucial role in biological experimentation. This has become especially true with regard to studies directed at understanding the fundamental genetic

35   and environmental factors associated with disease and the effects of potential therapeutic agents on the cell. Such a determination permits the early detection of infectious organisms such as bacteria, viruses, etc.; genetic diseases such as sickle cell anemia; and various cancers. New methods of diagnosis of diseases, such as AIDS, cancer, sickle cell

anemia, cystic fibrosis, diabetes, muscular dystrophy, and the like, rely on the detection of mutations present in certain nucleotide sequences. This paradigm shift has lead to an increasing need within the life science industries for more sensitive, more accurate and higher-throughput technologies for performing analysis on genetic material obtained

5   from a variety of biological sources.

Unique or misexpressed nucleotide sequences in a polynucleotide can be detected by hybridization with a nucleotide multimer, or oligonucleotide, probe. Hybridization reactions between surface-bound probes and target molecules in solution may be used to detect the presence of particular biopolymers. Hybridization is based on

10   complementary base pairing. When complementary single stranded nucleic acids are incubated together, the complementary base sequences pair to form double stranded hybrid molecules. These techniques rely upon the inherent ability of nucleic acids to form duplexes via hydrogen bonding according to Watson-Crick base-pairing rules. The ability of single stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) to

15   form a hydrogen bonded structure with a complementary nucleic acid sequence has been employed as an analytical tool in molecular biology research. An oligonucleotide probe employed in the detection is selected with a nucleotide sequence complementary, usually exactly complementary, to the nucleotide sequence in the target nucleic acid. Following hybridization of the probe with the target nucleic acid, any oligonucleotide probe/nucleic

20   acid hybrids that have formed are typically separated from unhybridized probe. The amount of oligonucleotide probe in either of the two separated media is then tested to provide a qualitative or quantitative measurement of the amount of target nucleic acid originally present.

Such reactions form the basis for many of the methods and devices used in the

25   field of genomics to probe nucleic acid sequences for novel genes, gene fragments, gene variants and mutations. The ability to clone and synthesize nucleotide sequences has led to the development of a number of techniques for disease diagnosis and genetic analysis. Genetic analysis, including correlation of genotypes and phenotypes, contributes to the information necessary for elucidating metabolic pathways, for understanding biological

30   functions, and for revealing changes in genes that confer disease. Many of these techniques generally involve hybridization between a target nucleotide sequence and a complementary probe, offering a convenient and reliable means for the isolation,

2

identification, and analysis of nucleotides. The surface-bound probes may be oligonucleotides, peptides, polypeptides, proteins, antibodies or other molecules capable of reacting with target molecules in solution.

Direct detection of labeled target nucleic acid hybridized to surface-bound polynucleotide probes is particularly advantageous if the surface contains a mosaic of different probes that are individually localized to discrete, known areas of the surface. Such ordered arrays of probes are commonly referred to as 'biochip" arrays. Biochip arrays have become an increasingly important tool in the biotechnology industry and related fields. Substrate-bound biopolymer arrays, particularly oligonucleotide, DNA and RNA arrays, may be used in screening studies for determination of binding affinity and in diagnostic applications, e.g., to detect the presence of a nucleic acid containing a specific, known oligonucleotide sequence.

The pattern of binding by target molecules to biopolymer probe spots on the biochip forms a pattern on the surface of the biochip and provides desired information about the sample. Hybridization patterns on biochip arrays are typically read by optical means, although other methods may also be used. For example, laser light in the Agilent Technologies Inc. GeneArray Scanner excites fluorescent molecules incorporated into the nucleic acid probes on a biochip, generating a signal only in those spots on the biochip that have a target molecule bound to a probe molecule, thus generating an optical hybridization pattern. This pattern may be digitally scanned for computer analysis. Such patterns can be used to generate data for biological assays such as the identification of drug targets, single-nucleotide polymorphism mapping, monitoring samples from patients to track their response to treatment, and assess the efficacy of new treatments.

One type of a linear array is a one-dimensional array of features bound in a non-diffusive manner to a surface usually located on the inside of an enclosed microchannel. The order of the features identifies each feature, which allows selective identification of target molecules. One such linear array is disclosed in U.S. Patent No. 5,804,384 (Muller, *et al.*). The devices of Muller, *et al.*, consist of a tube containing a linear array of specific binding elements that each have capture probes specific for a target analyte.

Inadequate mixing is a particular problem in chemical and biological assays where very small samples of chemical, biochemical, or biological fluids are typically

involved. Inhomogeneous solutions resulting from inadequate mixing can lead to poor hybridization kinetics, low efficiency, low sensitivity, and low yield. With inadequate mixing, diffusion becomes the only means of transporting the reactants in the mobile phase to the interface or surface containing the immobilized reactants. In such a case, the

5 mobile phase can become depleted of reactants near the substrate as mobile molecules become bound to the immobile phase.

Methods for mixing relatively large volumes of fluids usually utilize conventional mixing devices that mix the fluids by shaking the container, by a rapid mechanical up and down motion, or by the use of a rocking motion that tilts the

10 container filled with the fluids in a back and forth motion. The conventional mixing methods normally cannot be utilized for small volumes of fluid such as thin films of fluids in capillary chambers because the capillary strength of the containment system often significantly exceeds the forces generated by shaking or rocking, thereby preventing or minimizing fluid motion in the film. This is because most or all of the

15 fluid is so close to the walls of the chamber that there is virtually no bulk phase so that surface interactions predominate.

Therefore, there remains a need in the art for efficient and effective methods and apparatus for conducting and measuring results of hybridization reactions, particularly those carried out in small chambers such as capillary chambers in which linear arrays are

20 housed.

SUMMARY OF THE INVENTION

One embodiment of the present invention is a device for conducting binding reactions. The device comprises two chambers in fluid communication, wherein one of

25 the chambers (larger chamber) has a volume that is greater than a volume of the other chamber (smaller chamber). The device also comprises an array of features in each of the two chambers.

Another embodiment of the present invention is a device for conducting hybridization reactions. The device comprises two chambers in fluid communication.

30 Each of the chambers has an interior with capillary dimensions wherein one of the chambers has at least one interior dimension that is larger (larger chamber) than at least one interior dimension of the other of the chambers (smaller chamber). Each of the

interiors comprises an array of features comprising biopolymer probes. At least some of the probes of the smaller chamber are directed to target molecules having expected concentrations in a sample solution that are equal to or greater than a predetermined value and at least some of the probes of the larger chamber are directed to target

5   molecules having expected concentrations that are less than the predetermined value. Optionally, the features of the larger chamber may comprise a greater number of individual biopolymer probes than features of the smaller chamber.

Another embodiment of the present invention is an apparatus for conducting hybridization reactions. The apparatus comprises a housing having an interior with

10   capillary dimensions. The interior comprises at least two chambers in fluid communication. One of the chambers has interior dimensions that are larger (larger chamber) than the interior dimensions of the other of the chambers (smaller chamber). The interior comprises an array of features comprising biopolymer probes. At least some of the probes of the smaller chamber are directed to target molecules having expected

15   concentrations in a sample solution that are equal to or greater than a predetermined value and at least some of the probes of the larger chamber are directed to target molecules having expected concentrations that are less than the predetermined value. Optionally, the features of the larger chamber may comprise a greater number of individual biopolymer probes than features of the smaller chamber. The apparatus also

20   comprises a detector capable of examining the array and, as such, is in communication with the housing.

Another embodiment of the present invention is a device comprising an elongated web comprising a linear array of biopolymer features wherein the linear array is from 1 to 5 features in width. The elongated web comprises at least two chambers in

25   fluid communication where the chambers each having an interior with capillary dimensions. One of the chambers has interior dimensions resulting in volume that is larger (larger chamber) than the volume resulting from the interior dimensions of the other of the chambers (smaller chamber). At least some of the probes of the smaller chamber are directed to target molecules having expected concentrations in a sample

30   solution that are equal to or greater than a predetermined value and at least some of the probes of the larger chamber are directed to target molecules having expected concentrations that are less than the predetermined value. Optionally, the features of the

larger chamber may comprise a greater number of individual biopolymer probes than features of the smaller chamber.

Another embodiment of the present invention is a method for conducting binding reactions. A sample is introduced into a device as described above. Analytes in the sample bind to the biopolymer probes during an incubation period wherein the sample in the housing is incubated under conditions for carrying out the binding reactions.

Another embodiment of the present invention is a method for conducting hybridization reactions. A sample comprising target molecules is incubated in a capillary environment such that a portion of the sample is in contact with an array of features comprising biopolymer probes that are directed to target molecules having expected concentrations in the sample that are equal to or greater than a predetermined value and a portion of the sample is in contact with an array of features comprising biopolymer probes that are directed to target molecules having expected concentrations in the sample that are less than a predetermined value.

BRIEF DESCRIPTION OF THE DRAWINGS

The following figures are included to better illustrate the embodiments of the devices and techniques of the present invention. The figures are not to scale and some parts of the figures may be exaggerated for the purpose of illustrating certain aspects or embodiments of the present invention.

Fig. 1 is a perspective view taken from the top of an embodiment of an apparatus in accordance with the present invention.

Fig. 2 is a perspective view of a portion of a chamber of the device of Fig. 1.

Fig. 3 is a perspective view of an embodiment of an apparatus in accordance with the present invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

Some embodiments of devices of the invention comprise two or more chambers that are in fluid communication. The chambers generally have interiors that have capillary dimensions. The interiors of the chambers comprise arrays of features that are involved in the detection of target molecules or analytes in a sample of interest.

Typically, the features comprise biopolymer probes. In some embodiments each feature comprises at least one biopolymer probe and typically several biopolymer probes. The chambers are designed to maximize signal obtained when target molecules or analytes of interest in a sample to be analyzed are incubated within the interiors of the chambers. As mentioned above, for a linear array in a capillary housing with no mixing of sample, diffusion becomes the only means of transporting the reactants in the mobile phase to the interface or surface containing the immobilized reactants.

In embodiments of the present devices, the chambers have interiors that differ in dimensions such that at least one of the dimensions of one of the chambers is smaller (the "smaller chamber") than at least one of the dimensions of the other of the chambers (the "larger chamber"). The terms "smaller chamber" and "larger chamber" are used herein in the relative sense, i.e., the volume of one chamber relative to the other chamber. The dimensions, and thus the volume, of the smaller chamber are designed to constrain the amount of available target molecules that may be sensed by the biopolymer probes of the features in the interior of the smaller chamber. The dimensions, and thus the volume, of the larger chamber are designed to allow more target molecules to be sensed by the biopolymer probes of the features in the interior of the larger chamber.

In accordance with embodiments of the present invention, the dynamic range of a measurement of the results of reactions involving target molecules and the biopolymer probes may be extended by having biopolymer probes in at least some of the features of the smaller chamber that are capable of generating a higher signal level than that generated from biopolymer probes in at least some of the features of the larger chamber. Accordingly, the dimensions of the chambers may be designed to balance out variations in signal level. The result is a larger dynamic range for a given detector. Typically, the chambers are adjacent one another and more typically the chambers are contiguous with one another. The differing factor is that the dimensions or volume of one area of a conduit is larger than another area of the conduit so that a smaller chamber and a larger chamber may be realized to achieve the results discussed herein.

To further optimize signal level, the number of biopolymer probe molecules per feature (or feature size) may be varied. Larger features, i.e., features with higher

7

biopolymer probe concentrations, can be employed to accelerate reactions such as hybridization reactions as well as alter the sensitivity of a given feature. Accordingly, at least some of the features in the interior of the smaller chamber may be larger than features in the interior of the larger chamber. The terms "smaller features" and "larger features" are used herein in the relative sense, i.e., the size of one feature relative to the size of another feature.

In some embodiments of the present invention, some of the features of the smaller chamber are duplicates of features of the larger chamber. Accordingly, in such embodiments, some of the features in the smaller chamber have biopolymer probes that are the same as the biopolymer probes of features of the larger chamber. In this manner, signals between the two chambers may be mapped. The number of duplicate features is dependent on such considerations as feature signal repeatability, the desired accuracy of the mapping, the linearity of the mapping, the dynamic range of the mapping. and so forth. Typically, the number of duplicate features is about 1 to about 10,000, about 4 to about 1000, about 10 to about 400, about 20 to about 200, and so forth.

The chambers typically comprise all or part of a channel within a housing. The channel often has a capillary dimension, i.e., a cross-sectional area that provides for capillary flow through the channel. In general, for capillary channels or chambers, at least one of the cross-sectional dimensions, e.g., width, height, diameter, is at least about 1 μm, usually at least about 10 μm, and is usually no more than about 500 μm, typically no more than about 200 μm. Channels of capillary dimension typically have an inside bore diameter (ID) of from about 1 to about 200 microns, more typically from about 25 to about 100 microns.

The volume of the smaller chamber of embodiments of the present devices is dependent on a number of factors such as, for example, the concentration of the target molecules in a sample to be analyzed, the affinity of the biopolymer probes for the target molecules of interest, the size of the features comprising the biopolymer probes, whether the solution is mixed or not, and any other factors known to those in the art that influence hybridization rate, etc. The smaller chamber has cross-sectional dimensions consistent with the central concept of embodiments of the present invention.

Accordingly, as mentioned above, at least one of the dimensions, usually, cross-sectional dimensions, of the smaller chamber is chosen to achieve a volume in the smaller chamber sufficient to constrain the amount of available target molecules that may be sensed by the biopolymer probes of the features in the interior of the smaller chamber. The dimensions of the smaller chamber include length, width, height and diameter and the cross-sectional dimensions include width, height and diameter. The smaller chamber may be smaller by virtue of one or more of the dimensions being smaller than dimensions of the larger chamber. Typically, the smaller dimension is width or height or diameter. For example, for chambers that have a circular or oval interior shape, the width or diameter of the chamber is smaller than the width or diameter of the larger chamber. For chambers that have a rectangular or square interior shape, the width or height or both is smaller than the corresponding dimension of the larger chamber. Typically, the dimensions of the smaller chamber with a circular interior shape are about 1 to about 10,000 microns, about 5 to about 5,000 microns, about 10 to about 1,000 microns, about 20 to about 500 microns, about 20 to about 200 microns, and so forth, in diameter. Smaller chambers with non-circular interior shapes would have dimensions similar to the circular counterparts.

At least some of the biopolymer probes of the smaller chamber are directed to target molecules having expected concentrations in a sample solution that are equal to or greater than a predetermined value or cut-off value. The predetermined value is dependent on a number of factors such as, for example, scanner sensitivity, PMT gain on the scanner, dye choice, and the like. Essentially anything that affects the dynamic range of the scanner may influence the predetermined value.

As mentioned above, at least some of the biopolymer probes of the smaller chamber are directed to target molecules having expected concentrations in a sample solution that are equal to or greater than a predetermined value or cut-off value. The proportion of probes that are in this category versus other probes is dependent on considerations such as the type of sample being analyzed, the desired sensitivity one wants on the low expressed genes, and so forth. Typically, the proportion of probes that is equal to or greater than the predetermined value is 1 to about 90 %, about 5 to about

80 %, about 10 to about 80 %. The remaining probes may be probes directed to target molecules without regard for the predetermined value or probes directed to target molecules where the expected concentration is less than the predetermined value. Thus, some of the probes in the smaller chamber may be those that are also found in the larger chamber. The considerations for the proportion of probes in the larger chamber that are directed to target molecules having expected concentrations in a sample solution that are less than a predetermined value are similar to the considerations above for the smaller chamber.

The terms "higher concentration" of target molecules or components in a sample and "lower concentration" of target molecules or components in a sample are used herein in the relative sense. Accordingly, "higher" and "lower" in this sense are relative to one another, i.e. each of multiple features in the larger chamber (for example, most but not necessarily all the features in the larger chamber) bind to a target molecule or component of higher concentration than does each of multiple features in the smaller chamber (for example, most but not necessarily all the features in the smaller chamber).

The dimensions of the larger chamber of embodiments of the present devices are relative to the dimensions of the smaller chamber and are dependent on a number of factors such as those set forth above. Typically, at least one dimension of the larger chamber is about 110 to about 10,000 % larger, about 110 to about 1000 % larger, about 110 to about 500 % larger, than the dimension of the smaller chamber. The larger chamber has dimensions, usually, cross-sectional dimensions, consistent with the central concept of embodiments of the present invention. Accordingly, the dimensions of the larger chamber are chosen so as to provide a lesser constraint on the amount of available target molecules that may be sensed by the biopolymer probes of the features in the interior of the smaller chamber than that provided by the smaller chamber. Typically, the dimensions of the larger chamber that has a circular interior shape are about 10 to about 100,000 microns, about 50 to about 50,000 microns, about 100 to about 10,000 microns, about 200 to about 5,000 microns, in diameter. Larger chambers with non-circular interior shapes would have dimensions similar to the circular counterparts.

The array is typically a linear array and, as mentioned above, is generally present in a channel that permits capillary forces to act upon fluid in or around the channel. The channel may be partially or fully enclosed as long as such capillary forces may be realized. The channel may be in a housing, which may be a microchannel. The housing is any enclosure in which at least a portion of the interior has capillary dimensions. In one approach, the microchannel is part of a microfluidic system. Microfluidic systems have been developed for performing chemical, clinical, and environmental analysis of chemical and biological specimens. The term microfluidic system refers to a system or device having a network of chambers connected by channels, in which the channels have microscale features, that is, features too small to examine with the unaided eye. The term "microfluidic" generally means of or pertaining to fluids and being of a magnitude on the order consistent with capillary dimension. The channel(s) may be part of a microfluidic network or a system of interconnected cavity structures and capillary-size channels configured with a plurality of branches through which fluids may be manipulated and processed. However, in its simplest form, the present apparatus comprise a microchannel in the form of a tube within a housing.

In general, the material for the housing should provide physical support for the chemical compounds that are deposited on an interior surface of the housing or synthesized on an interior surface of the housing *in situ* from subunits. The materials should be of such a composition that they endure the conditions of a deposition process and/or an *in situ* synthesis and of any subsequent treatment or handling or processing that may be encountered in the use of a particular array.

Typically, the housing material is transparent or comprises a viewing area that is transparent. By "transparent" is meant that the substrate material permits signal from features on an interior surface of the substrate to pass therethrough without substantial attenuation and also permits any interrogating radiation to pass therethrough without substantial attenuation. By "without substantial attenuation" may include, for example, without a loss of more than about 40% or more typically without a loss of more than about 30%, about 20% or about 10%, of signal. The interrogating radiation and signal may for example be visible, ultraviolet or infrared light. In certain embodiments, such as for example where production of binding pair arrays for use in research and related applications is desired, the materials from which the substrate may be fabricated should

ideally exhibit a low level of non-specific binding during chemical reactions such as, e.g., hybridization events. Alternatively, the material may be opaque if the covering forming the top of channel comprising the linear array is removed or opened prior to a scanning for optical signal or if non-optical detection methods are employed such as
5   radiation.

Particular plastics finding use for the housing include, for example, flexible or rigid forms of polyethylene, polypropylene, polytetrafluoroethylene (PTFE), e.g., TEFLON®, polymethylmethacrylate, polycarbonate, polyethylene terephthalate, polystyrene or styrene copolymers, polyurethanes, polyesters, polycarbonates, polyureas,
10   polyamides, polyethyleneamines, polyarylene sulfides, polysiloxanes, polydimethylsiloxanes, polyimides, polyacetates, poly etheretherketone (PEEK), and the like, either used alone or in conjunction with another material or materials. The housing may be rigid or flexible.

Suitable rigid materials may include glass, which term is used to include silica,
15   and include, for example, glass such as glass available as Bioglass, and suitable rigid plastics and resins, and so forth. Rigid plastics include, for example, polymers such as, e.g., poly (vinyl chloride), polyacrylamide, polyacrylate, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), etc., either used by themselves or in conjunction with other
20   materials.

Microfluidic systems are often fabricated using photolithography, wet chemical etching, and other techniques similar to those employed in the semiconductor industry. The resulting devices can be used to perform a variety of sophisticated chemical and biological analytical techniques.

25   In one specific embodiment of the present apparatus, the channel is a conduit by which a sample may contact a linear array comprising a plurality, for example, array, of features for conducting chemical reactions. The channels, and thus the linear array, may be straight, curved, serpentine, labyrinth-like or other convenient configuration comprised of separate tubes or part of a monolithic, often planar, substrate. Although
30   the cross-sectional dimensions of the chambers of embodiments of the present devices are important, the cross-sectional shape of the channel is not critical and may be circular, ellipsoid, square, rectangular, triangular and the like. The inside of the channel may be

coated with a material for strength, for enhancing or reducing electrokinetic flow, for enhancing detection limits and sensitivity, and so forth. Exemplary of coatings are silylation, polyacrylamide (vinyl bound), methylcellulose, polyether, polyvinylpyrrolidone, and polyethylene glycol, polypropylene, Teflon™ (DuPont),

5 Nafion™ (DuPont), and the like may also be used.

The channel usually comprises at least one entry port, namely, any site at which a liquid may be introduced into a device having one or more channels. The entry port may be a well or simply the terminus of a channel that opens any place on the device such as at an edge. The channel usually comprises at least one port from which fluid exiting the

10 channel may travel to a collection chamber and the like.

Microfluidic devices generally include one or more channels fabricated on or within the devices, usually within the devices. The devices also can include reservoirs, fluidly connected to the channels, which can be used to introduce materials into the channels to contact a linear array contained in the channel. Microfluidic systems have a

15 number of advantages over conventional chemical or physical laboratory techniques. For example, microfluidic systems are particularly well adapted for analyzing small sample sizes, typically making use of samples on the order of nanoliters and even picoliters. The substrates may be produced at relatively low cost, and the channels can be arranged to perform numerous specific analytical operations, including mixing, dispensing, valving,

20 reactions, detections, electrophoresis, and the like. The analytical capabilities of such microfluidic systems may be enhanced by increasing the number and complexity of network channels, reaction chambers, and the like. However, in its simplest form for the purposes of the present invention involving linear arrays, the microfluidic system is often less complex.

25 The length of the linear array as manufactured is usually a fixed length determined by the number of features of the linear array. The number of features is related to the nature of the features, the nature of the analytes, the complexity of the biological or clinical questions being investigated, the number of quality control features desired, and so forth. A typical linear array may contain more than about ten, more than

30 about one hundred, more than about one thousand, more than about ten thousand, more than about twenty thousand, etc., more than about one hundred thousand, features and so forth.

13

The housing comprising the linear array may be prepared in a number of ways. The following discussion is by way of illustration and not limitation. In one approach, the linear array is synthesized or deposited on an interior surface of a housing substrate and the area comprising at least the linear array is enclosed to form a channel comprising

5 the linear array where the channel comprises at least two chambers as described above. Enclosure may be attained using an appropriate material to cover the channel and then sealing to form the housing. An apparatus may be fabricated using other convenient means, including conventional molding and casting techniques, extrusion sheet forming, calendaring, thermoforming, and the like. For example, with apparatus prepared from a

10 plastic material, a silica mold master, which is negative for the network structure in the planar substrate of one plate can be prepared by etching or laser micromachining. In addition to having a raised ridge, which forms the channel in the substrate, the silica mold may have a raised area that provides for one or more cavity structures including at least two chambers as described above in the planar substrate. Next, a polymer

15 precursor formulation can be thermally cured or photopolymerized between the silica master and support planar plate, such as a glass plate.

In one embodiment, a linear array may be synthesized or deposited on the surface of a flexible material or substrate in the dimensions desired. This embodiment is well suited for preparation of embodiments of an apparatus in accordance with the invention

20 comprising two chambers as described above. For example, for a microarray the chemical compounds comprising the linear array are synthesized or deposited in an area that corresponds to capillary dimensions where the area includes the aforementioned at least two chambers. The flexible substrate may be substantially flat along the area of synthesis or deposition or there may be a groove, depression, or the like in the housing

25 substrate where the linear array is placed. A portion or section of the flexible substrate comprises a flared section that serves as the larger chamber. The area of deposition or synthesis is ultimately enclosed to form a channel having the linear array therein. See, for example, U.S. Patent Application Serial No. 10/037757, entitled "Chemical Arrays" by Schembri, *et al.*, filed Oct. 18, 2001, published as U.S. Patent Publication No.

30 20030108726 and U.S. Patent Application Serial No. 10/032608, entitled "Chemical Arrays", by Lefkowitz, *et al.*, filed Oct. 18, 2001, published as U.S. Patent Publication No. 20030077380, the disclosures of which are incorporated herein by reference.

Enclosing the housing to form the channel comprising the linear array and the at least two chambers may be accomplished in a number of ways. One important consideration in forming the linear array housing in general, and enclosing the housing in particular, is to avoid damage to the linear array on the surface of the housing

5   substrate. In one approach, for example, the substrate is a flexible material that is folded over to enclose the housing to form the channel including the at least two chambers. In this approach the flexible substrate has a flared section corresponding generally to the larger chamber referred to above. After folding, the flexible material is sealed to itself in an area outside the area of the channel and outside the area that forms the interior of the

10   larger chamber. Sealing may be achieved by application of heat, adhesives, and so forth.

In an alternate approach, a separate material may be placed over the substrate comprising the linear array and the flared portion. The separate material is sealed to the substrate to enclose the housing to form the channel with the linear array therein and to form the at least two chambers. The separate material may be sealed to the substrate as

15   discussed above. The separate material may have the same composition as the substrate or a composition that is different from the substrate. The separate material may be flexible or rigid since the substrate to which it is sealed is flexible.

In a specific embodiment the present apparatus is manufactured from a flexible substrate, which may be the same as or similar to that described in U. S. Patent

20   Application No. 20030108726, filed October 18, 2001. The flexible substrate is a plastic, that is, any synthetic organic polymer of high molecular weight (for example at least 1,000 grams/mole, or even at least 10,000 or 100,000 grams/mole.

In one embodiment in accordance with the above disclosure, the flexible substrate may have a number of different layers. A base layer forms the greatest

25   thickness and may consist of any flexible plastic such as a polyolefin film (such as polypropylene, polyethylene, polymethylpentene) or polyetheretherketone, polyimide, any of the fluorocarbon polymers or other suitable flexible thermoplastic polymer film. The material of the base layer is best selected to provide stable dimensional, mechanical, and chemical properties as well as severability. For example, for polynucleotide arrays

30   the flexible substrate is subject to elevated temperatures (for example, 60°C) for long times (for example, 12 hours) in aqueous environments. Polyester or aramid films exposed to such conditions may tend to swell or degrade. When the type of linear arrays

and the conditions to which the layer will be exposed are selected, the base layer can be selected for dimensional, mechanical and chemical stability under such conditions by reference to many known polymer film characteristic sources. The base layer will typically have a thickness of more than about 1 μm (or more than about 5 μm) and less

5 than about 500 μm (or even less than about 100, about 50, about 25, or about 15 μm).

The flexible substrate may also include an optional reflective layer and a transparent layer. The reflective layer may be aluminum, silver, gold, platinum, chrome or other suitable metal film deposited by vacuum deposition, plasma enhanced chemical vapor deposition or other means onto the base layer or an optional intermediate bonding

10 layer. Alternatively, the reflective layer may be constructed using multiple dielectric layers designed as a dielectric Bragg reflector or the like. Typically, such a reflector is constructed by repeating ¼ wave thick layers of two optically clear dielectric materials that have differing indices of refraction. Design considerations for such a reflector include the excitation and emission wavelengths and the angle of incidence for the

15 excitation beam and detector. Although many of the materials from which the reflective layer may be made are rigid at a certain thickness, the thickness of the reflective layer for purposes of the present invention is such that the overall flexible substrate is sufficiently flexible. Accordingly, the thickness of the reflective layer is usually less than about 50 nm, or even less than about 20, about 10, about 5 or about 1 nm but in any

20 case, for example, more than about 0.1 or about 0.5 nm. The reflective layer may particularly be chosen to have a thickness such that it is opaque to the wavelength of the light used for illuminating the features during array reading.

A bonding layer, if used, may be any suitable material that is flexible at the thickness used and bonds to the base layer and/or the reflective layer. The bonding layer

25 may have a thickness of less than about 50 nm, or even less than about 20, about 10, about 5 or about 1 nm and usually more than about 0.1 or about 0.5 nm).

A glass layer (which term is used to include silica) may be deposited onto the reflective layer by sputtering, plasma enhanced chemical vapor deposition or similar techniques such as described in. A glass layer may optionally be used without a

30 reflective layer. Several manufacturers have commercial capabilities for providing films coated with metal and glass layers, for example, Sheldahl Corporation, Northfield, MN (see the world wide web site at www.sheldahl.com), and General Atomic, San Diego,

CA (world wide web site address of ga.com). The glass layer has a thickness such that the overall flexible substrate is sufficiently flexible. Accordingly, the thickness of the glass layer may be, for example, greater than about 1, about 10 or about 100 nm, and less than about 1000, about 700, or about 400 nm but typically has a thickness about ¼

5   wavelength of the light used to illuminate array features during reading, or an odd multiple of that amount. For example, about 40 to about 200 nm, or about 60 to about 120 nm (or even about 80 to about 100 nm), or an odd integer multiple of any of the foregoing thickness ranges (for example, about 300 nm may be used) provided the layer is not so thick that the flexible substrate is not sufficiently flexible.

10   The glass layer may particularly have a thickness and transparency selected as described in U.S. Patent Application Serial No. 09/493,958 titled "Multi-Featured Arrays With Reflective Coating" filed Jan. 28, 2000 by Andreas Dorsel, *et al.* while the reflective layer may meet the reflectivity requirements in relation to the illuminating light as mentioned in that application. For example, the reflective layer may reflect at

15   least about 10% of the incident light, or at least about 20%, about 50%, about 80% or at least about 90%, or even at least about 95%, of the incident light. As mentioned previously, this and the other references cited herein are incorporated into this application by reference.

In the above configuration of the flexible substrate, the use of a glass layer

20   allows the use of conventional chemistries, as discussed above, for substrate coating, feature fabrication, and array usage (for example, hybridization in the case of polynucleotide arrays). Such chemistries are well known for arrays on glass substrates, as described in the references cited herein and elsewhere. Furthermore, using a reflective layer not only can provide the useful characteristics mentioned in the above

25   referenced patent application Serial No. 09/493,958, but can avoid undesirable optical characteristics of the plastic base layer (for example, undesirable fluorescence, and in some instances, excessive heating and possible melting of the substrate). This allows for the ability to use base layers of a material that may have a high fluorescence and/or high absorbance of incident light. For example, the plastic base layer may have a

30   fluorescence of at least about five or about ten (or even at least: about twenty, about fifty, about one-hundred, or about two-hundred) reference units, and/or an absorbance of the illuminating light used to read arrays of at least about 5%, about 10%, about 20%, or

about 50% (or even at least about 70%, about 90% or about 95%).

Use of a non-reflective opaque layer (for example, a suitably dyed plastic or other layer) in place of reflective layer also allows the use of the foregoing materials for a base layer although in such a case some heat may then be generated in the opaque layer. A reflective or non-reflective opaque layer at the position of the base layer may block at least about 10% of the illuminating light incident on a front surface for reading arrays, and even at least about 20%, about 50%, or about 80% (or at least about 90% or about 95%) of the illuminating light. A non-reflective opaque layer may reflect less than about 95%, about 90%, about 80%, or about 50% (or even less than about 10%) of the illuminating light. Where neither a reflective layer nor other opaque layer is present, it will be preferable to employ a base layer that emits low fluorescence upon illumination with the excitation light, at least in the situation where the array is read by detecting fluorescence. The base layer in this case may emit less than about two hundred, about one hundred, about fifty, or about twenty (or even less than about ten or about five) reference units. Additionally in this case, the base layer is typically relatively transparent to reduce the absorption of the incident illuminating laser light and subsequent heating if the focused laser beam travels too slowly over a region. For example, the base layer may transmit at least about 5%, about 10%, about 20%, or about 50% (or even at least about 70%, about 90%, or about 95%), of the illuminating light incident on the front surface. Note that all reflection and absorbance measurements herein, unless the contrary is indicated, are made with reference to the illuminating light incident on a front surface for reading arrays and may be measured across the entire integrated spectrum of such illuminating light or alternatively at 532 nm or 633 nm.

An example of a specific embodiment of a device in accordance with the present invention is discussed next with reference to the attached drawings. Referring to Figs. 1 and 2, device 10 is depicted and comprises housing 12 with capillary chamber 14 and capillary chamber 16. Within chambers 14 and 16 are a linear array of features 18 (18a, 18b and 18c) diffusively bound to an interior surface 28 of housing 12. Entry port 22 is approximate one end of housing 12 and reservoir 24 is approximate an opposing end of housing 12. As can be seen, the width of capillary chamber 16 is larger than the width of capillary chamber 14. Features 18a in capillary chamber 14 are biopolymer probes that are directed to target molecules in a sample that are present at or above a predetermined

value. Features 18c in capillary chamber 14 are directed to target molecules that may be present in the sample with out regard for the predetermined value. Features 18b in capillary chamber 16 are biopolymer probes that are directed to target molecules in a sample that are present below a predetermined value. Features 18c in capillary chamber 5 16 are directed to target molecules that may be present in the sample with out regard for the predetermined value. Features 18c may be mapping probes as referred to hereinabove. Inter-feature regions 26 separate features 18.

A typical linear array may contain from about 100 to about 100,000 features. At least some, or all, of the features are of different compositions (for example, when any 10 repeats of each feature composition are excluded the remaining features may account for at least about 5%, about 10%, or about 20% of the total number of features). Each feature carries a predetermined moiety (such as a particular polynucleotide sequence), or a predetermined mixture of moieties (such as a mixture of particular polynucleotides).

In some embodiments a linear array of features is a one-dimensional array of 15 features bound in a non-diffusive manner to a surface. By the term "non-diffusive" is meant that the molecules that make up the individual features are bound to the surface in such a manner that they will not detach under the conditions of preparing and using the linear array. Non-diffusive binding may be covalent or may be non-covalent or macromolecular association where the linking is of sufficient strength to withstand the 20 aforementioned conditions. Non-diffusive binding of the features may be achieved in a number of approaches known in the art. Some of those approaches are discussed briefly hereinbelow by way of illustration and not limitation.

The features generally are molecules that are involved in the detection of target molecules or analytes in a sample of interest. Each molecule of a feature may be specific 25 for a corresponding analyte or for a compound indicative of the presence of the analyte. For example, the analyte may be part of a complex such as, for example, an antigen-antibody complex, polynucleotide-protein complex, polynucleotide-polynucleotide complex and the like, and the feature is capable of binding to a component of the complex. Usually, the molecule comprising the feature is a specific binding partner for 30 the analyte or for a member of the complex indicative of the presence of the analyte. The members of a pair of molecules (e.g., a detector probe or a capture probe and a target analyte, or the members of a specific binding pair (e.g., antibody-antigen, nucleic acid,

and protein-vitamin binding pairs)) are said to "specifically bind" to each other if they bind to each other with greater affinity than to other, non-specific molecules. For example, an antibody raised against an antigen to which it binds more efficiently than to a non-specific antigen can be described as specifically binding to the antigen. Similarly,

5   a nucleic acid probe can be described as specifically binding to a nucleic acid target if it forms a specific duplex with the target by base pairing interactions.

Each feature, or element, within the linear array is defined to be a small, regularly shaped region of the surface of the substrate. The features in the linear array are arranged in a predetermined manner. Each feature of a linear array usually carries a

10   predetermined chemical compound or mixtures thereof and is typically of homogeneous composition. Each feature within the linear array may contain a different molecular species, and the molecular species within a given feature may differ from the molecular species within the remaining features of the molecular array. Some or all of the features may be of different compositions. Each array may be separated by spaces or areas.

15   Interarray areas and interfeature areas are usually present but are not essential. These interarray and interfeature areas do not carry any chemical compound such as polynucleotide (or other biopolymer of a type of which the features are composed). Interarray areas and interfeature areas typically will be present where arrays are formed by the conventional *in situ* process or by deposition of previously obtained moieties, as

20   described herein, by depositing for each feature at least one droplet of reagent such as from a pulse jet but may not be present when, for example, photolithographic array fabrication processes are used. It will be appreciated though that the interarray areas and interfeature areas, when present, could be of various sizes and configurations.

In the linear array the order of the features identifies each feature, which allows

25   selective identification of target molecules. Usually, the linear array has a fixed length determined by the number of features of the linear array. The width of the linear array is usually one feature. However, for purposes of the present invention, the width of the linear array may be greater than one feature where the size of the feature and the width of the housing, e.g., microchannel, permit. Therefore, the width of the linear array may

30   be 1 to about 5 features, 1 to about 4 features, 1 to about 3 features, 1 to 2 features. In such an embodiment where the linear array is more than one feature wide, each feature comprising the width at the position in question may be the same or different and each

feature comprising the length of the linear array may be the same or different, usually different, as discussed above. The width of the features, for example, the diameter of a round spot, may be in the range from about 10 μm to about 1.0 cm. In other embodiments each feature may have a width in the range of about 1.0 μm to about 1.0

5   mm, usually about 5.0 μm to about 500 μm, and more usually about 10 μm to about 200 μm. Non-round features may have width ranges equivalent to that of circular features with the foregoing width (diameter) ranges.

The width of a feature may be larger, for example, where the feature comprises a higher concentration of probes than that of another feature. As mentioned above, further

10   optimization of signal level may be obtained in embodiments of the present invention by having features in, for example, the smaller chamber, that have a higher concentration of probes than features in the larger chamber. However, larger features may be employed in both chambers if desired.

In some embodiments of the present invention, some of the features of the

15   smaller chamber are duplicates of features of the larger chamber. Accordingly, in such embodiments, some of the features in the smaller chamber have biopolymer probes that are the same as the biopolymer probes of features of the larger chamber. In this manner, signals between the two chambers may be mapped, that is, the signals may be compared and a mathematical relationship derived describing how the signal levels of the two

20   chambers relate.

Furthermore, in some embodiments mapping probes are placed in both of the chambers in order to determine how much the signal is compressed. By "signal compression" is meant the amount the signal levels of the probes are attenuated by being located in the smaller chamber.

25   By "mapping probes" is meant probes that are located in both the smaller and larger chambers and are utilized to derive the mathematical relationship between the relative signal levels of similar probes between chambers.

The interior surface of the housing to which a plurality of chemical compounds is attached to form the linear array can be hydrophilic or capable of being rendered

30   hydrophilic or it may be hydrophobic. The interior surface is normally treated to create a primed or functionalized surface, that is, a surface that is able to support the attachment of a fully formed chemical compound or the synthetic steps involved in the production

of the chemical compound on the surface of the substrate. Functionalization relates to modification of the surface of a substrate to provide a plurality of functional groups on the substrate surface. By the term "functionalized surface" is meant a substrate surface that has been modified so that a plurality of functional groups are present thereon

5   usually at discrete sites on the surface. The manner of treatment is dependent on the nature of the chemical compound to be synthesized or deposited and on the nature of the surface. In one approach a reactive hydrophilic site or reactive hydrophilic group is introduced onto the surface of the substrate. Such hydrophilic moieties can be used as the starting point in a synthetic organic process.

10   The surface of the housing onto which the chemical compounds are deposited or formed may be modified with one or more different layers of compounds that serve to modify the properties of the surface in a desirable manner. Such modification layers, when present, will generally range in thickness from a monomolecular thickness to about 1 mm, usually from a monomolecular thickness to about 0.1 mm and more usually

15   from a monomolecular thickness to about 0.001 mm. Modification layers of interest include: inorganic and organic layers such as metals, metal oxides, polymers, small organic molecules and the like. Polymeric layers of interest include layers of: peptides, proteins, polynucleic acids or mimetics thereof (for example, peptide nucleic acids and the like); polysaccharides, phospholipids, polyurethanes, polyesters, polycarbonates,

20   polyureas, polyamides, polyethylene amines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, and the like, where the polymers may be hetero- or homo-polymeric, and may or may not have separate functional moieties attached thereto (for example, conjugated). Various further modifications to the particular embodiments described above are, of course, possible. Accordingly, the present invention is not

25   limited to the particular embodiments described in detail above.

As mentioned above, the chemical compounds that are bound to the interior surface of the housing to form the linear array may be synthesized or deposited on the surface. Usually, an initial derivatization of the surface is carried out. Modification of surfaces for use in chemical synthesis has been described. See, for example, U.S. Patent

30   No. 5,266,222 (Willis) and U.S. Patent No. 5,137,765 (Farnsworth).

The arrays may be, and are usually, microarrays created on the interior surface of the housing by *in situ* synthesis of biopolymers such as polynucleotides, polypeptides,

polysaccharides, etc., and combinations thereof, or by deposition of molecules such as oligonucleotides, cDNA and so forth. In general, arrays are synthesized on a surface by one of any number of synthetic techniques that are known in the art.

In one embodiment, the surface of the substrate is siliceous, i.e., the surface comprises silicon oxide groups, either present in the natural state or introduced by techniques well known in the art. One technique for introducing siloxyl groups onto the surface involves reactive hydrophilic moieties on the surface. These moieties are typically epoxide groups, carboxyl groups, thiol groups, and/or substituted or unsubstituted amino groups as well as a functionality that may be used to introduce such a group such as, for example, an olefin that may be converted to a hydroxyl group by means well known in the art. One approach is disclosed in U.S. Patent No. 5,474,796 (Brennan), the relevant portions of which are incorporated herein by reference. A siliceous surface may be used to form silyl linkages, i.e., linkages that involve silicon atoms. Usually, the silyl linkage involves a silicon-oxygen bond, a silicon-halogen bond, a silicon-nitrogen bond, or a silicon-carbon bond.

Another method for attachment is described in U.S. Patent No. 6,219,674 (Fulcrand, *et al.*). A surface is employed that comprises a linking group consisting of a first portion comprising a hydrocarbon chain, optionally substituted, and a second portion comprising an alkylene oxide or an alkylene imine wherein the alkylene is optionally substituted. One end of the first portion is attached to the surface and one end of the second portion is attached to the other end of the first portion chain by means of an amine or an oxy functionality. The second portion terminates in an amine or a hydroxy functionality. The surface is reacted with the substance to be immobilized under conditions for attachment of the substance to the surface by means of the linking group.

Another method for attachment is described in U.S. Patent No. 6,258,454 (Lefkowitz, *et al.*). A solid substrate having hydrophilic moieties on its surface is treated with a derivatizing composition containing a mixture of silanes. A first silane provides the desired reduction in surface energy, while the second silane enables functionalization with molecular moieties of interest, such as small molecules, initial monomers to be used in the solid phase synthesis of oligomers, or intact oligomers. Molecular moieties of interest may be attached through cleavable sites.

A procedure for the derivatization of a metal oxide surface uses an aminoalkyl silane derivative, e.g., trialkoxy 3-aminopropylsilane such as aminopropyltriethoxy silane (APS), 4-aminobutyltrimethoxysilane, 4-aminobutyltriethoxysilane, 2-aminoethyltriethoxysilane, and the like. APS reacts readily with the oxide and/or siloxyl groups on metal and silicon surfaces. APS provides primary amine groups that may be used to carry out the present methods. Such a derivatization procedure is described in EP 0 173 356 B1, the relevant portions of which are incorporated herein by reference. Other methods for treating the surface of a substrate to which the chemical compounds become bound will be suggested to those skilled in the art in view of the teaching herein.

The invention has particular application to linear arrays of oligomers or polymers. The oligomer or polymer is a chemical entity that contains a plurality of monomers. Polymers usually comprise at least two monomers but may comprise thousands of monomers, ten of thousands of monomers or more. Oligomers generally comprise about 6 to about 100 monomers. Examples of polymers include polydeoxyribonucleotides, polyribonucleotides, other polynucleotides that are C-glycosides of a purine or pyrimidine base, or other modified polynucleotides, polypeptides, polysaccharides, and other chemical entities that contain repeating units of like chemical structure. Exemplary of oligomers are oligonucleotides and peptides.

A monomer is a chemical entity that can be covalently linked to one or more other such entities to form an oligomer or polymer. Examples of monomers include nucleotides, amino acids, saccharides, peptoids, and the like and subunits comprising nucleotides, amino acids, saccharides, peptoids and the like. The subunits may comprise all of the same component such as, for example, all of the same nucleotide or amino acid, or the subunit may comprise different components such as, for example, different nucleotides or different amino acids. The subunits may comprise about 2 to about 2000, or about 5 to about 200, monomer units. In general, the monomers have first and second sites (e.g., C-termini and N-termini, or 5' and 3' sites) suitable for binding of other like monomers by means of standard chemical reactions (e.g., condensation, nucleophilic displacement of a leaving group, or the like), and a diverse element that distinguishes a particular monomer from a different monomer of the same type (e.g., an amino acid side chain, a nucleotide base, etc.). The initial substrate-bound, or support-bound, monomer is generally used as a building block in a multi-step synthesis procedure to form a

complete ligand, such as in the synthesis of oligonucleotides, oligopeptides, oligosaccharides, etc. and the like.

A biomonomer references a single unit, which can be linked with the same or other biomonomers to form a biopolymer (for example, a single amino acid or
5   nucleotide with two linking groups one or both of which may have removable protecting groups). A biomonomer fluid or biopolymer fluid reference a liquid containing either a biomonomer or biopolymer, respectively (typically in solution).

A biopolymer is a polymer of one or more types of repeating units. Biopolymers are typically found in biological systems and particularly include polysaccharides (such
10   as carbohydrates), and peptides (which term is used to include polypeptides, and proteins whether or not attached to a polysaccharide) and polynucleotides as well as their analogs such as those compounds composed of or containing amino acid analogs or non-amino acid groups, or nucleotide analogs or non-nucleotide groups. This includes polynucleotides in which the conventional backbone has been replaced with a non-
15   naturally occurring or synthetic backbone, and nucleic acids (or synthetic or naturally occurring analogs) in which one or more of the conventional bases has been replaced with a group (natural or synthetic) capable of participating in Watson-Crick type hydrogen bonding interactions.

A polynucleotides are compounds or compositions that are polymeric nucleotides
20   or nucleic acid polymers. The polynucleotide may be a natural compound or a synthetic compound. Polynucleotides include oligonucleotides and are comprised of natural nucleotides such as ribonucleotides and deoxyribonucleotides and their derivatives although unnatural nucleotide mimetics such as 2'-modified nucleosides, peptide nucleic acids and oligomeric nucleoside phosphonates are also used. The polynucleotide can
25   have from about 2 to 5,000,000 or more nucleotides. Usually, the oligonucleotides are at least about 2 nucleotides, usually, about 5 to about 100 nucleotides, more usually, about 10 to about 50 nucleotides, and may be about 15 to about 30 nucleotides, in length. Polynucleotides include single or multiple stranded configurations, where one or more of the strands may or may not be completely aligned with another.

30   A nucleotide refers to a sub-unit of a nucleic acid and has a phosphate group, a 5 carbon sugar and a nitrogen containing base, as well as functional analogs (whether synthetic or naturally occurring) of such sub-units which in the polymer form (as a

polynucleotide) can hybridize with naturally occurring polynucleotides in a sequence specific manner analogous to that of two naturally occurring polynucleotides. For example, a "polynucleotide" includes DNA (including cDNA), RNA, oligonucleotides, and PNA and other polynucleotides as described in US 5,948,902 and references cited therein (all of which are incorporated herein by reference), regardless of the source. An "oligonucleotide" generally refers to a nucleotide multimer of about 10 to 100 nucleotides in length, while a "polynucleotide" includes a nucleotide multimer having any number of nucleotides.

Embodiments of devices and methods of the present invention are particularly useful for carrying out binding reactions between biopolymers such as hybridization reactions between polynucleotides or binding reactions between poly(amino acids). Some embodiments of the present devices and methods are useful where the chemical compounds of the array are oligonucleotides and such oligonucleotide arrays are employed for determinations of polynucleotides.

As mentioned above, biopolymer arrays can be fabricated by depositing previously obtained biopolymers (such as from synthesis or natural sources) onto a substrate, or by *in situ* synthesis methods. The *in situ* synthesis methods include those described in U.S. Pat. No. 5,449,754 for synthesizing peptide arrays, as well as WO 98/41531 and the references cited therein for synthesizing polynucleotides (specifically, DNA). Such in situ synthesis methods can be basically regarded as repeating at each spot the sequence of: (a) deprotecting any previously deposited monomer so that it can now link with a subsequently deposited protected monomer; and (b) depositing a droplet of another protected monomer for linking. Different monomers may be deposited at different regions on the substrate during any one iteration so that the different regions of the completed array will have different desired biopolymer sequences. One or more intermediate further steps may be required in each iteration, such as oxidation, capping and washing steps. The deposition methods basically involve depositing biopolymers at predetermined locations on a substrate, which are suitably activated such that the biopolymers can link thereto. Biopolymers of different sequence may be deposited at different regions of the substrate to yield the completed array. Washing or other additional steps may also be used. Reagents used in typical in situ synthesis are water sensitive, and thus the presence of moisture should be eliminated or at least minimized.

The *in situ* method for fabricating a polynucleotide array typically follows, at each of the multiple different addresses at which features are to be formed, the same conventional iterative sequence used in forming polynucleotides from nucleoside reagents on a substrate by means of known chemistry. This iterative

5   sequence is as follows: (a) coupling a selected nucleoside through a phosphite linkage to a functionalized substrate in the first iteration, or a nucleoside bound to the substrate (i.e. the nucleoside-modified substrate) in subsequent iterations; (b) optionally, but typically, blocking unreacted hydroxyl groups on the substrate bound nucleoside; (c) oxidizing the phosphite linkage of step (a) to form a

10   phosphate linkage; and (d) removing the protecting group ("deprotection") from the now substrate bound nucleoside coupled in step (a), to generate a reactive site for the next cycle of these steps. The functionalized substrate (in the first cycle) or deprotected coupled nucleoside (in subsequent cycles) provides a substrate bound moiety with a linking group for forming the phosphite linkage with a next

15   nucleoside to be coupled in step (a). A number of reagents involved in the above synthetic steps such as, for example, phosphoramidite reagents, are sensitive to moisture and anhydrous conditions and solvents are employed. Final deprotection of nucleoside bases can be accomplished using alkaline conditions such as ammonium hydroxide, in a known manner.

20   The foregoing chemistry of the synthesis of polynucleotides is described in detail, for example, in Caruthers, Science 230: 281-285, 1985; Itakura, *et al.*, Ann. Rev. Biochem. 53: 323-356; Hunkapillar, *et al.*, Nature 310: 105-110, 1984; and in "Synthesis of Oligonucleotide Derivatives in Design and Targeted Reaction of Oligonucleotide Derivatives", CRC Press, Boca Raton, Fla., pages 100 *et seq.*, U.S.

25   Patent Nos. 4,458,066, 4,500,707, 5,153,319, and 5,869,643, EP 0294196, and elsewhere.

As mentioned above, various ways may be employed to produce an array of polynucleotides on the surface of a substrate. Such methods are known in the art. One *in situ* method employs pulse-jet technology to dispense the appropriate phosphoramidite

30   reagents and other reagents onto individual sites on a surface of a substrate. Oligonucleotides are synthesized on a surface of a substrate *in situ* using phosphoramidite chemistry. Solutions containing nucleotide monomers and other

reagents as necessary such as an activator, e.g., tetrazole, are applied to the surface of a substrate by means of thermal pulse-jet technology (although piezoelectric activated pulse jets might also be used, but in any event the pulse jets used must be constructed of materials chemically compatible with the solutions used). Individual droplets of reagents

5   are applied to reactive areas on the surface using, for example, a thermal pulse-jet type nozzle. The surface of the substrate may have an alkyl bromide trichlorosilane coating to which is attached polyethylene glycol to provide terminal hydroxyl groups. These hydroxyl groups provide for linking to a terminal primary amine group on a monomeric reagent. Excess of non-reacted chemical on the surface is washed away in a subsequent

10   step. For example, see U.S. Patent No. 5,700,637 and PCT WO 95/25116 and PCT application WO 89/10977.

Another approach for fabricating an array of biopolymers on a substrate using a biopolymer or biomonomer fluid and using a fluid dispensing head is described in U.S. Patent No. 6,242,266 (Schleifer, *et al.*). The head has at least one jet that can dispense

15   droplets onto a surface of a substrate. The jet includes a chamber with an orifice and an ejector, which, when activated, causes a droplet to be ejected from the orifice. Multiple droplets of the biopolymer or biomonomer fluid are dispensed from the head orifice so as to form an array of droplets on the surface of the substrate.

In another embodiment (U.S. Patent No. 6,232,072) (Fisher) a method of, and

20   apparatus for, fabricating a biopolymer array is disclosed. Droplets of fluid carrying the biopolymer or biomonomer are deposited onto a front side of a transparent substrate. Light is directed through the substrate from the front side, back through a substrate backside and a first set of deposited droplets on the first side to an image sensor.

An example of another method for chemical array fabrication is described in

25   U.S. Patent No. 6,180,351 (Cattell). The method includes receiving from a remote station information on a layout of the array and an associated first identifier. A local identifier is generated corresponding to the first identifier and associated array. The local identifier is shorter in length than the corresponding first identifier. The addressable array is fabricated on the substrate in accordance with the received layout information.

30   The housing for the linear array may include an identifier, which is generated and used as described in U.S. Patent No. 6,180,351 titled "Chemical Array Fabrication with Identifier". The identifiers may also optionally include a communication address, which

identifies the address of a remote location on communication channel from which one or more characteristics of an array will be communicated in response to a received communication of the associated identifier. Such remote location may be that of communication module or alternatively that of another accessible memory on a

5   communication channel carrying the database of array characteristic data and associated identifiers. Examples of a communication address may be a telephone number, computer ID on a WAN, or an internet Universal Resource Locator.

Devices and apparatus of the present invention may be employed in various assays involving biopolymers. An exemplary embodiment of a use of a device of the

10   invention will be discussed with reference to Fig. 1. A sample is introduced into housing 12 through entry port 22 of device 10 as described above. Features 18a in chamber 14 comprise oligonucleotide probes directed to target polynucleotides having an expected concentration in the sample that is equal to or greater than predetermined value 0.01 pM. Features 18b in chamber 16 comprise oligonucleotide probes directed to target

15   polynucleotides having an expected concentration in the sample that is less than predetermined value 0.01 pM. Features 18c are duplicate features within chamber 14 and chamber 16 and are employed for the purposes discussed above. The sample is typically drawn within chambers 14 and 16 by means of capillary action.

For purposes of this example, the linear array comprises 500 features at a spacing

20   of 100 microns. Smaller chamber 14 is 200 microns in width and 30 microns in depth and larger chamber 16 is 2,000 microns in width and 30 microns in depth. The sample is one suspected of containing the analyte(s) of interest (for example, a fluorescent-labeled polynucleotide or protein-containing sample). After the linear array has been exposed to the sample and after a sufficient incubation period, the array is then read. Intervening

25   washing steps may be employed to remove unbound materials prior to reading of the array.

The sample may be a trial sample, a reference sample, a combination of the foregoing, or a known mixture of components such as polynucleotides, proteins, polysaccharides and the like (in which case the arrays may be composed of features that

30   are unknown such as polynucleotide sequences to be evaluated). The samples may be from biological assays such as in the identification of drug targets, single-nucleotide polymorphism mapping, monitoring samples from patients to track their response to

treatment and/or assess the efficacy of new treatments, and so forth. For hybridization reactions the sample generally comprises a target molecule that may or may not hybridize to a surface-bound molecular probe. The term "target molecule" refers to a known or unknown molecule in a sample, which will hybridize to a molecular probe on

5   a substrate surface if the target molecule and the molecular probe contain complementary regions. In general, the target molecule is a "biopolymer," i.e., an oligomer or polymer. The present devices and methods have particular application to various processing steps involved with the aforementioned hybridization reactions.

An oligonucleotide probe may be, or may be capable of being, labeled with a

10   reporter group, which generates a signal, or may be, or may be capable of becoming, bound to one a feature of the linear array. Detection of signal depends upon the nature of the label or reporter group. Commonly, binding of an oligonucleotide probe to a target polynucleotide sequence is detected by means of a label incorporated into the target. Alternatively, the target polynucleotide sequence may be unlabeled and a second

15   oligonucleotide probe may be labeled. Binding can be detected by separating the bound second oligonucleotide probe or target polynucleotide from the free second oligonucleotide probe or target polynucleotide and detecting the label. In one approach, a sandwich is formed comprised of one oligonucleotide probe, which may be labeled, the target polynucleotide and an oligonucleotide probe that is or can become bound to a

20   surface of a support. Alternatively, binding can be detected by a change in the signal-producing properties of the label upon binding, such as a change in the emission efficiency of a fluorescent or chemiluminescent label. This permits detection to be carried out without a separation step. Finally, binding can be detected by labeling the target polynucleotide, allowing the target polynucleotide to hybridize to a surface-bound

25   oligonucleotide probe, washing away the unbound target polynucleotide and detecting the labeled target polynucleotide that remains. Direct detection of labeled target polynucleotide hybridized to surface-bound oligonucleotide probes is particularly advantageous in the use of ordered arrays.

In one approach, cell matter is lysed, to release its DNA as fragments, which are

30   then separated out by electrophoresis or other means, and then tagged with a fluorescent or other label. The DNA mix is exposed to the linear array of oligonucleotide probes, whereupon selective attachment to matching probe sites takes place. The array is then

washed and the result of exposure to the array is determined. In this particular example, the array is imaged by scanning the surface of the support so as to reveal for analysis and interpretation the sites where attachment occurred.

The signal referred to above may arise from any moiety that may be incorporated into a molecule such as an oligonucleotide probe for the purpose of detection. Often, a label is employed, which may be a member of a signal producing system. The label is capable of being detected directly or indirectly. In general, any reporter molecule that is detectable can be a label. Labels include, for example, (i) reporter molecules that can be detected directly by virtue of generating a signal, (ii) specific binding pair members that may be detected indirectly by subsequent binding to a cognate that contains a reporter molecule, (iii) mass tags detectable by mass spectrometry, (iv) oligonucleotide primers that can provide a template for amplification or ligation and (v) a specific polynucleotide sequence or recognition sequence that can act as a ligand such as for a repressor protein, wherein in the latter two instances the oligonucleotide primer or repressor protein will have, or be capable of having, a reporter molecule and so forth. The reporter molecule can be a catalyst, such as an enzyme, a polynucleotide coding for a catalyst, promoter, dye, fluorescent molecule, chemiluminescent molecule, coenzyme, enzyme substrate, radioactive group, a small organic molecule, amplifiable polynucleotide sequence, a particle such as latex or carbon particle, metal sol, crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, a mass tag that alters the weight of the molecule to which it is conjugated for mass spectrometry purposes, and the like.

The signal may be produced by a signal producing system, which is a system that generates a signal that relates to the presence or amount of a target polynucleotide in a medium. The signal producing system may have one or more components, at least one component being the label. The signal producing system includes all of the reagents required to produce a measurable signal. The signal producing system provides a signal detectable by external means, by use of electromagnetic radiation, desirably by visual examination. Signal-producing systems that may be employed in the present invention are those described more fully in U.S. Patent Nos. 6,558,908, 6,251,588, 6,235,483 and 6,132,997, the relevant disclosure of which is incorporated herein by reference.

31

The features of the linear array and the liquid sample are maintained in contact for a period of time sufficient for the desired chemical reaction to occur. The conditions for a reaction, such as, for example, period of time of contact, temperature, pH, salt concentration and so forth, are dependent on the nature of the chemical reaction, the

5   nature of the chemical reactants including the liquid samples, and the like. The conditions for binding of members of specific binding pairs are generally well known and will not be discussed in detail here. The conditions for the various processing steps are also known in the art.

The linear arrays prepared as described above are particularly suitable for

10   conducting hybridization reactions. Such reactions are carried out on a substrate or support comprising a plurality of features relating to the hybridization reactions. The substrate is exposed to liquid samples and to other reagents for carrying out the hybridization reactions. The support surface exposed to the sample is incubated under conditions suitable for hybridization reactions to occur.

15   After an appropriate period of time of contact between the liquid sample and the linear array (incubation period), the contact is discontinued and various processing steps are performed. Following the processing step(s), the linear array is examined by an examining device. The linear array may be moved to an examining device where the linear array is interrogated or the examining device may be part of an apparatus

20   comprising the device of the present invention. The examining device may be a scanning device involving an optical system. Reading of the array may be accomplished by any suitable detector such as, for example, CCD, CMOS detector, photomultiplier tube (PMT), and the like. As mentioned above, because of the higher dynamic range provided by embodiments of the present invention, inexpensive detectors such as, e.g., CCD and

25   CMOS detectors, may be employed.

In some embodiments reading of the array may be accomplished by illuminating the array and reading the location and intensity of resulting fluorescence at each feature of the array. For example, a scanner may be used for this purpose where the scanner may be similar to, for example, the AGILENT MICROARRAY SCANNER available

30   from Agilent Technologies Inc, Palo Alto, CA. Other suitable apparatus and methods are described in U.S. patent applications: Serial No. 09/846,125 "Reading Multi-Featured Arrays" by Dorsel, *et al.*; and U.S. Patent No. 6,406,849. The relevant portions

of these references are incorporated herein by reference. However, arrays may be read by methods or apparatus other than the foregoing, with other reading methods including other optical techniques (for example, detecting chemiluminescent or electroluminescent labels) or electrical techniques (where each feature is provided with an electrode to

5   detect hybridization at that feature in a manner disclosed in U.S. Patent Nos. 6,221,583 and 6,251,685, and elsewhere).

As mentioned above, the examining device may be part of an apparatus comprising devices in accordance with the present invention. An embodiment of such an apparatus is depicted in Fig. 3. Apparatus 100 comprises tray 102 having a plurality of

10   devices 10 retained on surface 108 of tray 102. Apparatus 100 further comprises examining device 114, which is mounted on base plate 110 and includes a motor 116 for moving robotic arm 118 to which examining device 114 is attached. Computer 120 is in communication with motor 116 and examining device 114. Results read from the linear arrays are communicated to computer 120. In this way, examining device 114 is capable

15   of examining the array and, as such, is in examining communication with the housing of device 10.

Results from the reading may be raw results (such as fluorescence intensity readings for each feature in one or more color channels) or may be processed results such as obtained by rejecting a reading for a feature that is below a predetermined

20   threshold and/or forming conclusions based on the pattern read from the array (such as whether or not a particular target sequence may have been present in the sample). The results of the reading (processed or not) may be forwarded (such as by communication) to a remote location if desired, and received there for further use (such as further processing).

25   When one item is indicated as being "remote" from another, this means that the two items are at least in different buildings and may be at least one mile, ten miles, or at least one hundred miles apart. "Communicating" information references transmitting the data representing that information as electrical signals over a suitable communication channel (for example, a private or public network). "Forwarding" an

30   item refers to any means of getting that item from one location to the next, whether by physically transporting that item or otherwise (where that is possible) and includes, at

least in the case of data, physically transporting a medium carrying the data or communicating the data.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were

5   specifically and individually indicated to be incorporated by reference, except insofar as they may conflict with those of the present application (in which case the present application prevails). Methods recited herein may be carried out in any order of the recited events, which is logically possible, as well as the recited order of events.

The aforementioned description includes theories and mechanisms by which the

10   invention is thought to work. It should be noted, however, that such proposed theories and mechanisms are not required and the scope of the present invention should not be limited by any particular theory and/or mechanism.

Although embodiments of the foregoing invention have been described in some detail by way of illustration and example for purposes of clarity of understanding, it will

15   be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. Furthermore, the foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the invention. However, it will be appreciated that one skilled in the

20   art that the specific details are not required in order to practice the invention. Thus, the foregoing descriptions of specific embodiments of the present invention are presented for purposes of illustration and description; they are not intended to be exhaustive or to limit the invention to the precise forms disclosed. Many modifications and variations are possible in view of the above teachings. The embodiments were chosen and described

25   in order to explain the principles of the invention and its practical applications and to thereby enable others skilled in the art to utilize the invention.